

# Modulation of attention and stress with arousal: the mental and physical effects of riding a motorcycle

## Supplemental Online Materials

### Hormonal assay procedure

Methods from Supplemental Material (Nave et al., 2017).

Salivary steroids (testosterone, DHEA) were measured by LC-MS/MS using an AB Sciex Triple Quad 5500. Internal standards were added to 1 ml of saliva and the steroids then extracted by C18 column chromatography with 0.1 M NH<sub>4</sub>OH wash followed by 10% acetone. Steroids were eluted from the SPE with 10% methanol in acetone and dried under nitrogen. The dried samples were subjected to derivatization—the process of transforming a compound into a derivative product of similar chemical structure—with pyridine-3-sulfonyl chloride for the estrogens (estrone (E1), estradiol (E2), and estriol (E3)) as outlined by **Xu and Spink** (Xu & Spink, 2008). 40 µL sodium bicarbonate (50mM, pH 10) and 40 µL pyridine-3-sulfonyl chloride (3 mg/mL in acetonitrile) were added to the dried samples, and incubated at 60°C for 10 minutes. After derivatization, the samples were diluted with 80 µL of water and injected for LC-MS/MS analysis with analytical separation performed on an Agilent Poroshell 120 EC-C8 column and ionization by atmospheric pressure chemical ionization (APCI) in the positive ionization mode. Table S2 lists each analyte along with its validation results for the lower limit of quantitation (LLOQ is jargon for the lowest level of detection with coefficients of variation (CVs) < 20% over the linear range), linear range, and the inter-assay precision from the highest concentration to the LLOQ within the linear range. When salivary hormone levels of participants were below their LLOQ, we assigned values halfway between zero and their respective LLOQ (note that the true quantities of the hormone in the sample are never zero, even when they do not reach the detection threshold). Cortisol and epinephrine were gathered from urine and normalized for kidney output, a method with an R<sup>2</sup> of 0.98 compared to standard liquid collection practices (Zava et al., 2013).

### References

- Nave, G., Nadler, A., Zava, D., & Camerer, C. (2017). Single-Dose Testosterone Administration Impairs Cognitive Reflection in Men. *Psychological Science*, 28(10), 1398–1407.  
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Zava, T. T., Kapur, S., & Zava, D. T. (2013). Iodine and creatinine testing in urine dried on filter paper. *Analytica Chimica Acta*, 764, 64–69. <https://doi.org/10.1016/j.aca.2012.12.021>